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Actin-dependent Cell Elongation in Teleost Retinal Rods: Requirement for Actin Filament Assembly

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ABSTRACT Teleost retinal rods elongate when exposed to light. Elongation is mediated by a narrow necklike region called the myoid. In the cichlid *Sarotherodon mossambicus*, the rod inner segment (composed of the myoid with adjacent ellipsoid) increases in length from 12 μm in the dark to 41 μm in the light. Long light-adapted myoids contain longitudinally oriented microtubules and bundles of parallel 60-Å filaments that we have identified as actin by their ability to bind myosin subfragment 1. In short dark-adapted myoids, only microtubules are recognizable. Colchicine experiments reveal that light-induced rod elongation can occur in the absence of myoid microtubules. Intraocular injections of colchicine at concentrations that disrupt virtually all rod myoid microtubules do not block rod elongation. However, rod elongation is blocked by intraocular injections of cytochalasin B or cytochalasin D. The hierarchy of effectiveness of these drugs is consistent with their effectiveness in inhibiting actin assembly and in disrupting other actin-dependent motile processes. On the basis of ultrastructural observations and the results of these inhibitor studies, we propose that the forces responsible for rod elongation are dependent not on microtubules but on actin filament assembly.

Both microtubules and actin filaments have been implicated in cell shape changes. The participation of microtubules in cell elongation and in the maintenance of cell shape is generally accepted (8, 12, 19). Evidence for the role of microtubules in these processes is usually of two sorts: first, elongating cells often contain microtubules oriented parallel to the axis of elongation (8, 11, 26); second, shape changes can be blocked by agents such as colchicine, which result in the disassembly of microtubules (12, 27). Although in most cases microtubules are required for elongation to occur, it is not yet clear whether they produce force or act as supportive struts.

Actin and actin-associated proteins clearly play a role in the extension of certain cell projections (21, 23, 30) and in generating certain cell shape changes (13). Rapid polymerization of F-actin appears to generate the force required to extend the acrosomal process of sea cucumber sperm (23). In other instances, the lateral cross-linking of filaments is thought to generate the forces that produce cell shape change. When sea urchin coelomocytes change shape from a petaloid to a filopodial form, their actin filaments undergo lateral aggregation into stiff bundles (13). These actin filament bundles eventually form the core of the filopodia and hence presumably act as cytoskeletal elements. In *Limulus* sperm, the acrosomal process appears to extend by a different mechanism; force production

is thought to be a consequence of changes in the packing arrangement of preexisting actin filaments (21). In all of these cases, microtubules are not present within the extended cell process.

We report here a previously undescribed example of cell elongation, the teleost retinal rod, in which force production appears to depend on an actin-based mechanism rather than a microtubule-based mechanism. Teleost rods elongate in the light and contract in the dark. These movements are part of the daily cycle of photoreceptor and pigment epithelial movements, called retinomotor movements, which adjust the retina morphologically to bright and dim light (2). By elongating in the light, the rods bury their light-sensitive outer segments in the shielding pigment of the retinal pigmented epithelium.

Elongation is mediated by the delicate necklike region of the rod called the myoid. Rod movements provide useful models for a structural characterization of elongation because the movements are uniaxial and the diameter of the myoid is very small (2.9 μm in the dark-adapted state). Rod myoids are structurally simple: they contain parallel bundles of actin filaments, a few longitudinally oriented microtubules, one or two slender tubules of smooth endoplasmic reticulum, and virtually nothing else. We have attempted, by using appropriate inhibitors, to discriminate between the relative contributions of

microtubules and actin filaments to light-induced elongation and to the maintenance of the elongated shape.

MATERIALS AND METHODS

Animals

Three species of fish are used in this report: the cichlid, *Sarotherodon mossambicus*; the green sunfish, *Lepomis cyanellus*; and the killifish, *Fundulus heteroclitus*. *Sarotherodon mossambicus* were used for the morphological and the inhibitor studies because the arrangement of rods into a single tier in this fish facilitates quantitation of changes in cell length. Green sunfish were used for negative-stain preparations, and *Fundulus heteroclitus* were used for the subfragment (S1) binding experiments. In the course of other studies, rods have been examined in many additional species of teleosts. With the exception of *Cichlasoma citrinellum* (where the rods are relatively stationary; cf. reference 15), all the species we have examined exhibit similar rod morphology and retinomotor movements.

Fish were maintained in outdoor ponds under ambient light conditions. Light-adapted fish were exposed to normal fluorescent room lights (860–970 lx) for at least 2 h before any experiment. Fish were dark adapted by placing them in an aerated dark tank for at least 2.5 h. Eyes of dark-adapted fish were removed as quickly as possible under dim red light.

Electron Microscopy

Retinas were fixed either by immersion fixation or by perfusion fixation. For immersion fixation, eyes were enucleated and the front half of the eyecup including the lens was dissected away. Either the posterior half with the retina *in situ* or the isolated retina was immediately immersed in fixative at room temperature. Eyecups were fixed overnight, whereas isolated retinas were fixed for 20–30 min. Some isolated retinas were treated with 0.05% saponin in a solution containing 0.15 M KCL, 10 mM phosphate buffer (pH 7.0), 15 mM $MgCl_2$, and 0.2 mM dithiothreitol (DTT) for 4 min before immersion. Perfusion fixation procedures have been previously described (10).

The fixative contained 1% glutaraldehyde (TAAB EM grade; TAAB Laboratories, Reading, England) in 0.1 M phosphate or 0.1 M cacodylate (pH 7.0), 1 mM $MgCl_2$, and 0.2% tannic acid. Postfixation was carried out in 1% OsO_4 for 0.75–1.0 h on ice. Retinas were *en bloc* stained with 0.1% aqueous uranyl acetate for 45 min in the dark. Dehydration and embedding procedures have been described elsewhere (10).

Myosin S1 Decoration

Procedures for S1 decoration have been described previously (10).

Negative Staining

Procedures for retinal cell dissociation and negative staining of detergent-disrupted cells have been described previously by Burnside (9).

Rod Length Measurements

Rod inner segment length was determined by measuring the distance from the outer limiting membrane (OLM) to the base of the rod outer segment. Mean lengths were calculated by measuring a minimum of 15 rod inner segments from at least two different fish.

Inhibitor Studies

Colchicine was dissolved in L-15 culture medium (Grand Island Biological Co., Grand Island, N. Y.) and injected intraocularly to give a calculated final concentration of 4 mM. Cytochalasins B and D were dissolved in 0.75–1.5% dimethyl sulfoxide (DMSO) in L-15 medium and injected to give calculated final concentrations (see below) of 3, 4, and 6 $\mu g/ml$. One eye was injected with the drug and the other was injected with the carrier medium of the drug (L-15 or L-15 \pm DMSO). Concentrations were calculated by assuming dilution to total eye volume. Two groups of two fish each were used: (a) dark-adapted transferred to light and (b) light-adapted left in light. 3 h after injection, fish were sacrificed and retinas were fixed.

Preparation of Lumicolchicine

Lumicolchicine was prepared by irradiating an aqueous solution of colchicine (6.25×10^{-4} M) with unfiltered focused light from an Illumination Industries (Sunnyvale, Calif.) 100 W high-pressure mercury arc. Irradiation continued until the absorbance of colchicine at 355 nm decreased from 0.6 to 0.05. To obtain a

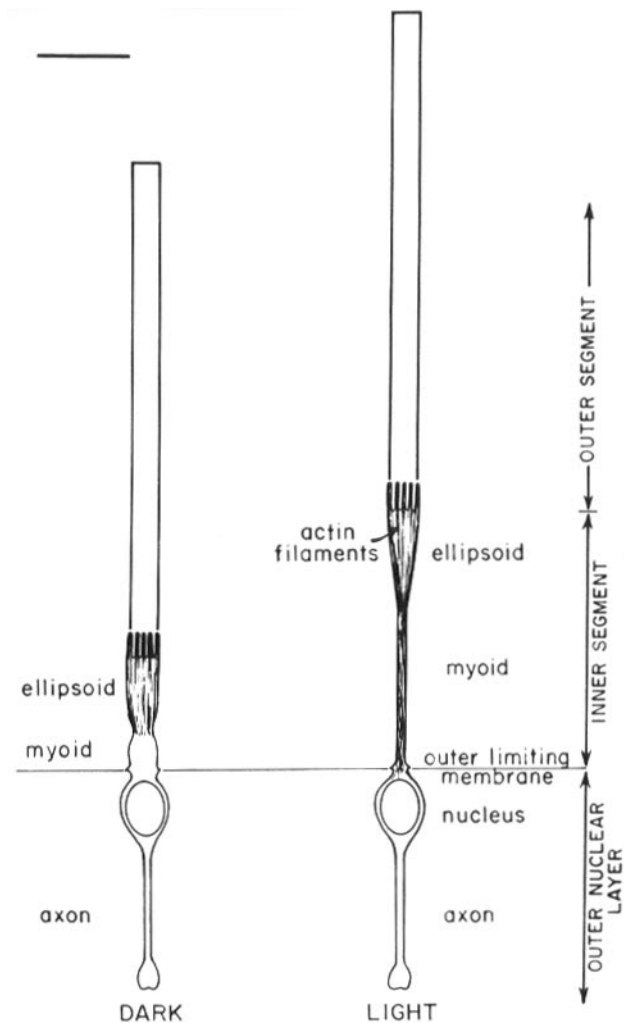
higher concentration for injection, the lumicolchicine solution was lyophilized and rediluted with L-15 buffer to a 0.1 M final concentration.

RESULTS

Morphology of the Rod Cell

Elongation in teleost retinal rods is primarily limited to the necklike myoid region of the cell (Fig. 1). In the dark-adapted state, the myoid of the rod is a short broad trunk with the ellipsoid lying close to the OLM (Fig. 2a and b). The OLM contains Müller (glial) cell processes that form adherens junctions with both rods and cones. Those regions of the cell that are distal to the OLM (outer segment, ellipsoid, myoid) are free to move, whereas those regions lying proximal are stationary (Fig. 1).

Upon exposure to light, the rod myoid extends to become a long slender stalk (Fig. 3a and b). Inner segment length (measured from the OLM to the base of the outer segment) increases from 12.5 ± 0.1 (mean \pm SE; $n = 30$) to 41.3 ± 0.8 ($n = 30$) during light adaptation of *Sarotherodon mossambicus*. The inner segment includes both the myoid and the mitochondria-filled region called the ellipsoid. Although the ellipsoid



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FIGURE 1 Schematic illustration of cell shape and thin filament distribution in dark- and light-adapted rods. Not shown are the ellipsoid and the central myoid microtubules that are present in both states. Bar, 10 μm .

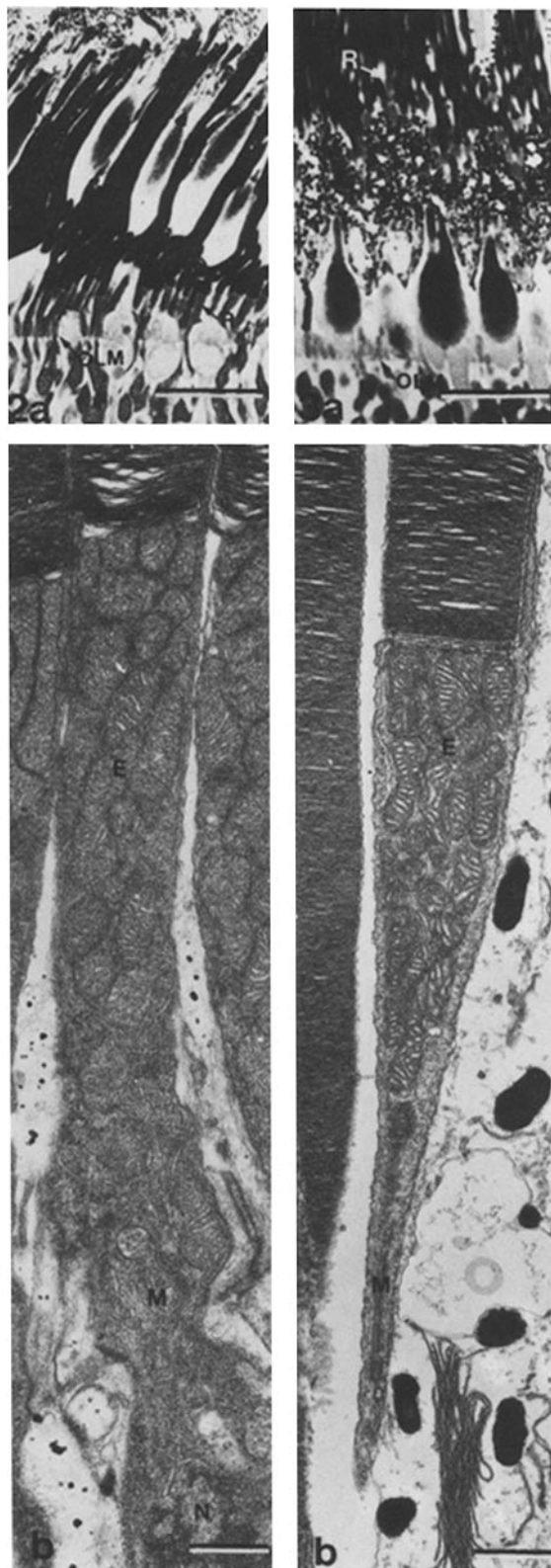


FIGURE 2 (a) Light micrograph of a retina from a cichlid fish, *Sarotherodon mossambicus*, fixed after 2 h in the dark. Rod ellipsoids (R) are positioned close to the outer limiting membrane (OLM). Bar, 10 μm . $\times 515$. (b) Electron micrograph of a dark-adapted rod from the retina of a *S. mossambicus*. The myoid (M) is the short broad region of the cell lying between the ellipsoid (E) and the nucleus (N). Bar, 1 μm . $\times 12,000$.

FIGURE 3 (a) Light micrograph of a retina from a light-adapted *S. mossambicus*. Rod myoids are fully elongated. Rod ellipsoid (R),

extends slightly by becoming more conical, $>94\%$ of the length increase occurs in the myoid. As the rod elongates, the mean diameter of the myoid decreases from $2.9 \mu\text{m} \pm 0.2$ ($n = 15$) in the dark-adapted state to $0.26 \mu\text{m} \pm 0.01$ ($n = 36$) in the light-adapted state.

Thin Filaments of the Rod Cell

Fig. 1 schematically illustrates the distribution of thin filaments in dark- and light-adapted rods. We have examined several species of teleosts and find that all but catfish, which have much larger rod myoids, exhibit similar filament distribution (cf. Materials and Methods). 60-Å filaments are visible in thin sections (Fig. 4a and b) as well as in negatively stained preparations of detergent-disrupted rods (Fig. 5a and b). Both ellipsoid and myoid filaments bind myosin S1 to yield arrowheads characteristic of actin filaments (Fig. 4c).

Bundles of 60-Å filaments originate from the tips of the calyceal processes (microvillus-like projections that encompass the base of the outer segment) and extend down along the perimeter of the ellipsoid. In the long light-adapted rod, the filaments of the ellipsoid bundles continue into the myoid either as a circumferential ring of paraxial filaments underlying the myoid membrane or as a central core bundle. We have not been able to detect regularly spaced bridges between myoid filaments or between membrane and myoid filaments.

Ellipsoid filament bundles appear unchanged in light- and dark-adapted states. However, myoid filaments are not observed in contracted dark-adapted rods even with fixation conditions that clearly preserve filaments in the long light-adapted rods.

Microtubules of the Rod Cell

In addition to the parallel 60-Å filaments, the rod myoid contains a few longitudinally oriented microtubules. The microtubules lie in the core of the myoid and are surrounded by the myoid filaments. In long light-adapted rods, the number of microtubules seen in a cross section is $2.1 \mu\text{m} \pm 0.2$ ($n = 30$).

Effect of Colchicine on Rod Elongation

Intraocular injections of colchicine at concentrations that disrupt myoid microtubules do not block light-induced rod elongation (Fig. 6a and b). Rods of colchicine-injected eyes elongate to the same length attained by rods in the contralateral eye injected only with buffer (Table I), even though virtually all rod myoid microtubules are disrupted in the colchicine-injected eye (Table II and Fig. 7).

The final concentration of colchicine used (4 mM) was estimated by assuming dilution to full eye volume: this estimate is almost certainly high because of aqueous humor outflow and leakage. The concentration used was selected because in a dose/response survey it was the lowest concentration that completely blocked dark-induced cone elongation in the fish we used (*Sarotherodon*). Cone elongation has previously been shown to be microtubule dependent (27).

It should be noted from Table I that although, in the colchicine-injected eye, rods elongate as much as in the contralateral buffer-injected eye, neither of these eyes exhibits the

outer limiting membrane (OLM). Bar, 10 μm . $\times 515$. (b) Electron micrograph of a light-adapted rod from the retina of a *S. mossambicus*. The myoid (M) of the rod has elongated into a long slender stalk. E, ellipsoid. Bar, 1 μm . $\times 12,000$.

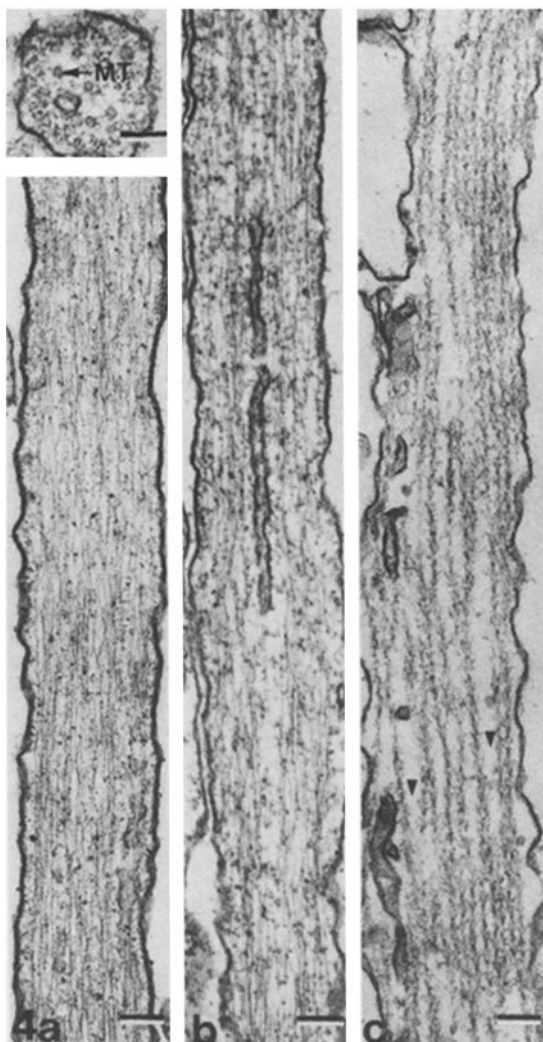


FIGURE 4 (a) Longitudinal and cross sections (inset) through the rod myoid of a light-adapted green sunfish, *Lepomis cyanellus*. Peripheral thin filaments surround the core of microtubules (MT). Although the myoids of this fish are longer and have more numerous microtubules, the filament organization is the same as in *S. mossambicus*. Bar, 0.1 μm . $\times 60,000$. (b) Longitudinal section of rod myoid thin filaments from light-adapted *S. mossambicus*. Bar, 0.1 μm . $\times 60,000$. (c) Myosin S1 decoration of a light-adapted *Fundulus heteroclitus* rod myoid. Filaments form typical arrowhead complexes (arrows) that predominantly point toward the nucleus. Bar, 0.1 μm . $\times 60,000$.

maximal elongation observed in uninjected fish. In fact, rods in colchicine-injected fish elongate to only $\sim 59\%$ the normal maximal length. We were puzzled by this result because in the colchicine-injected eye microtubules were absent, whereas in the contralateral buffer-injected eye the microtubules were not significantly different from those in rods of uninjected fish (Table II). Because the buffer- and the buffer + DMSO-injected contralateral eyes in cytochalasin-injected fish achieve maximal length (Table III), we are confident that impaired movement does not result from injection damage to the eye.

By carrying out parallel experiments using lumicolchicine, a photoderivative of colchicine that is not capable of binding tubulin (3, 29), we have investigated the possibility that a microtubule-independent effect of colchicine might be responsible for this partial inhibition of elongation. Lumicolchicine produces the same partial inhibition of elongation as colchicine

(Table I) and yet fails to disrupt microtubules in the rod myoids (Table II). Thus it seems likely that the partial inhibition of rod elongation we observe is a microtubule-independent effect that occurs at a colchicine concentration much lower than that required for microtubule disruption. Hence, the impairment of elongation in the contralateral eye probably results from systemic colchicine derived secondarily from the colchicine-injected eye. To further support this hypothesis, we examined the effect of unilateral colchicine injections on dark-induced cone elongation. In the colchicine-injected right eye, cone elongation is completely inhibited, whereas in the contralateral buffer-injected eye, cones exhibit partial elongation, i.e., myoids extend to $18.8 \pm 0.1 \mu\text{m}$ ($n = 30$) as compared with $30.1 \pm 1.3 \mu\text{m}$ ($n = 30$) in the uninjected fish. Thus we conclude that there is leakage of colchicine into the systemic circulation, which exposes the buffer-injected contralateral eye to lower colchicine concentrations.

Effect of Cytochalasins B and D on Rod Elongation

Intraocular injections of cytochalasins B and D strongly inhibit light-induced rod elongation (Fig. 8 *a* and *b*; Table III). As has been observed in other motile systems (17, 20), cytochalasin D is more effective than cytochalasin B (Table III). In cytochalasin-blocked rods, no myoid thin filaments are visible; i.e., their ultrastructure is indistinguishable from that of dark-adapted rods (Fig. 9 *a* and *b*). Inhibition of rod elongation is reversible after 48 h (Table III).

Maintenance of the Elongated Shape

Neither colchicine nor cytochalasin induces shortening of long rods in the absence of light change (Table IV). Colchicine was used at a concentration that was previously shown to disrupt rod myoid microtubules. Thus shortening of the rod cannot be attributed merely to the collapse of myoid microtubules.

We were unable to evaluate the role of myoid actin filaments in the maintenance of the elongated shape because cytochalasin B treatments failed to disrupt myoid filaments. The highest concentration we used was 6 $\mu\text{g}/\text{mg}$ (calculated final concentration)—a dosage that blocks rod myoid elongation by 92%. Clearly, the filament apparatus of the myoid, once assembled, is insensitive to cytochalasin.

DISCUSSION

Previous morphological descriptions (1, 25) of teleost retinal rods report the presence of microtubules within the myoid region of the cell. In *Sarotherodon mossambicus* and several other fish, we have demonstrated that 60-Å filaments, as well as microtubules, are present within the rod myoid. We have identified these 60-Å filaments as actin by their ability to bind myosin S1, resulting in the formation of arrowhead complexes (14). In this paper, we report that light-induced rod elongation is independent of myoid microtubule participation but does require the assembly and possibly the cross-linking of actin filaments.

Microtubules in Rod Elongation

Microtubules are present in both the dark- and light-adapted states of the rod myoid. Rods treated with colchicine, a drug known to bind tubulin (6, 28) and to disassemble microtubules (12), elongate when exposed to light, even though virtually all

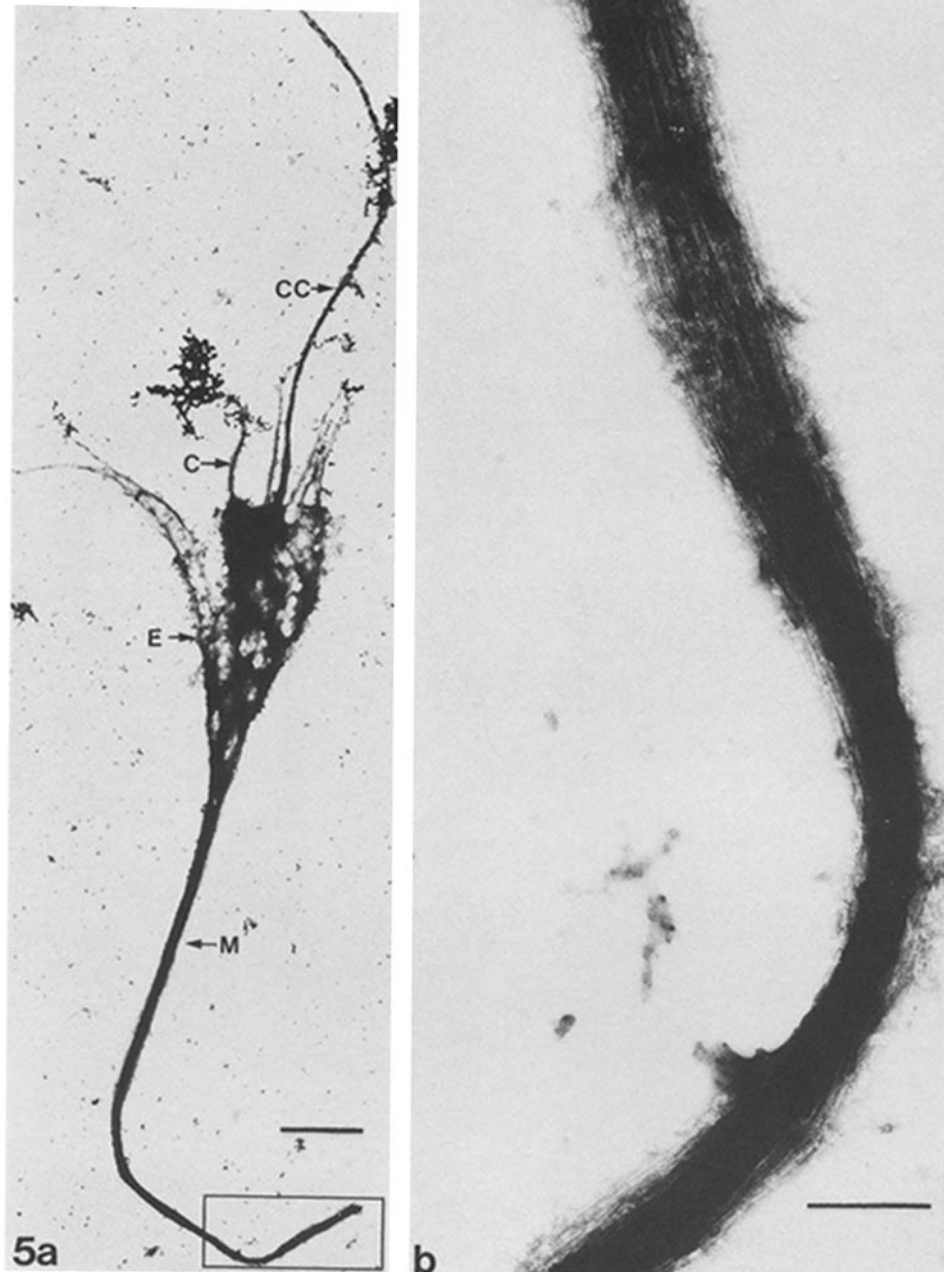


FIGURE 5 (a) Negatively stained preparation of the cytoskeleton of a rod from a light-adapted green sunfish. The rods were sheared from the retina by gentle vortexing, placed on an EM grid, disrupted with Triton X-100, and stained with uranyl acetate. Calyceal process (C), connecting cilium (CC), ellipsoid (E), myoid (M). Bar, $0.2 \mu\text{m}$. $\times 6,000$. (b) High magnification of actin filaments from the myoid region of the rod (box in a). Bar, $0.2 \mu\text{m}$. $\times 90,000$.

microtubules within the myoids are disrupted. This colchicine concentration blocks dark-induced cone elongation in the same fish. Cone elongation has previously been shown to be colchicine sensitive in other fish species (27).

Although significant light-induced rod elongation occurs in the presence of colchicine, maximum light-adapted length is not attained. Because lumicolchicine also results in a partial blockage of light-induced elongation without disrupting micro-

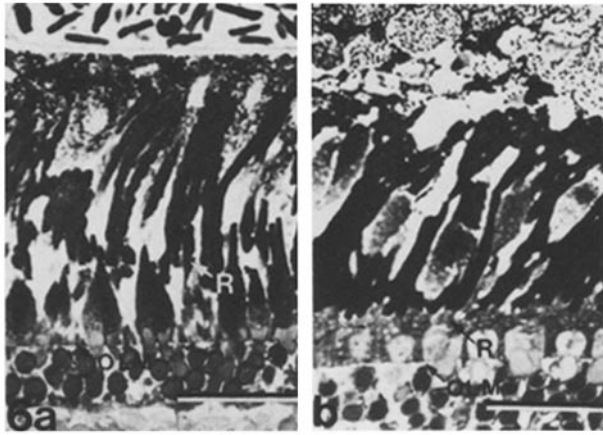


FIGURE 6 (a) Light micrograph of retina from a dark-adapted *S. mossambicus* injected with L-15 buffer and transferred to light. Rods elongate to $\sim 2.5 \times$ their original length. Rod ellipsoid (R), outer limiting membrane (OLM). Bar, $10 \mu\text{m}$. $\times 515$. (b) Light micrograph of retina from contralateral eye of dark-adapted *S. mossambicus* injected with colchicine (calculated final concentration, 4 mM) and transferred to light. Rods elongate to same length as rods of control eye. Rod ellipsoid (R), outer limiting membrane (OLM). Bar, $10 \mu\text{m}$. $\times 515$.

TABLE I
Effect of Colchicine and Lumicolchicine on Rod Elongation

	Rod inner segment length $\mu\text{m} \pm$	n *
Initial dark-adapted state	12.5 ± 0.1	30
Light-adapted state	41.3 ± 0.8	30
Colchicine (calculated final concentration, 4 mM)	29.6 ± 0.5	60
Colchicine control (L-15 buffer)	28.9 ± 0.4	50
Lumicolchicine (calculated final concentration, 4 mM)	34.7 ± 1.1	30
Lumicolchicine control (L-15 buffer)	33.7 ± 0.6	30

* n, Number of rods.

\pm Mean \pm SE.

TABLE II
Effect of Colchicine and Lumicolchicine on Myoid Microtubules

	No of Microtubules/ myoid*	n
Uninjected light-adapted state	2.1 ± 0.2	30
Colchicine (calculated final concentration, 4 mM)	0.5 ± 0.2	30
Colchicine control (L-15 buffer)	2.3 ± 0.2	36
Lumicolchicine (calculated final concentration, 4 mM)	1.6 ± 0.1	57

* Mean \pm SE.

tubules, we conclude that the partial blockage produced by colchicine results from a microtubule-independent effect of the drug. We also note that this partial blockage occurs at a lower concentration of colchicine than is required to disrupt microtubules. Similar results on lens epithelial cell elongation have been obtained by Beebe et al. (4). They report that colchicine, at concentrations lower than those that dissociate microtubules,

blocks lens cell elongation possibly by inhibiting an increase in cell volume (4).

In additional studies, to be reported elsewhere, we have observed that colchicine blocks rod contraction. This surprising result suggests that microtubules could be involved in rod contraction. Further investigation of this possibility is in progress.

Actin Filaments in Rod Elongation

Our ultrastructural observations suggest that actin filaments are assembled during rod elongation. No thin filaments are visible within the myoid of short dark-adapted rod cells, but long light-adapted myoids do contain numerous thin filaments. Possible sites of monomer addition during filament assembly could be at the distal tip of the calyces, at the free proximal ends of the ellipsoid filaments, or at points along the length of the filaments. The latter possibility seems unlikely because it would require breaking several monomer-monomer bonds. Because the distal ends of the filaments appear to be embedded in the dense tip of the calyceal process, we think it more likely

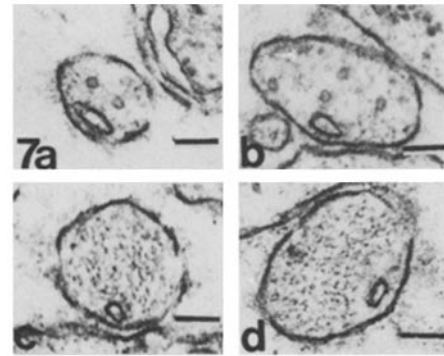


FIGURE 7 (a and b) Electron micrographs of cross sections of rod myoids from *S. mossambicus* injected with L-15 and transferred to light. Note the presence of microtubules. Bar, $0.1 \mu\text{m}$. $\times 60,000$. (c and d) Electron micrographs of cross sections of rod myoids from *S. mossambicus* injected with colchicine (calculated final concentration, 4 mM) and transferred to light. Microtubules are absent. Bar, $0.1 \mu\text{m}$. $\times 60,000$.

TABLE III
Effect of Cytochalasin on Rod Elongation

	Rod inner segment length* μm	n \pm
Controls		
Initial dark-adapted state	12.5 ± 0.1	30
Light-adapted state (uninjected)	41.3 ± 0.8	30
Injected eye + L-15 buffer	38.2 ± 0.6	60
Injected eye + L-15 buffer + DMSO	37.5 ± 0.9	45
Experimental, calculated final concentration		
Cytochalasin B, $3 \mu\text{g/ml}$	37.5 ± 1.3	30
Cytochalasin B, $4 \mu\text{g/ml}$	25.3 ± 0.6	30
Cytochalasin D, $4 \mu\text{g/ml}$	13.2 ± 0.3	30
Cytochalasin B, $6 \mu\text{g/ml}$	15.8 ± 0.5	30
Recovery, calculated final concentration		
Cytochalasin D, $4 \mu\text{g/ml}$	$29.7 \pm 0.7 \pm$	30

* Mean \pm SE.

\pm 48 h after injection.

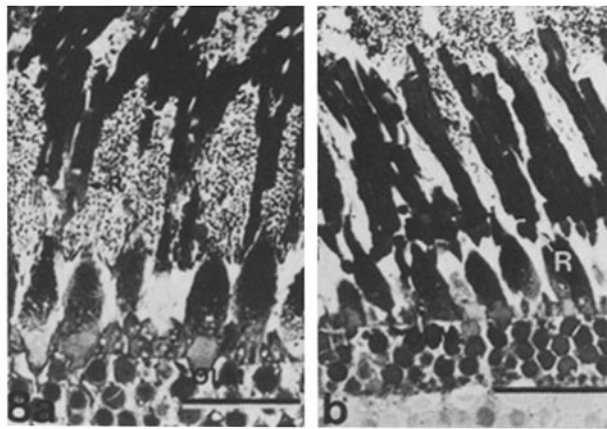


FIGURE 8 (a) Light micrograph of a retina from a dark-adapted *S. mossambicus* injected with L-15 buffer and transferred to light. Rods elongate normally. Rod ellipsoid (R), outer limiting membrane (OLM). Bar, 10 μ m. \times 515. (b) Light micrograph of a retina from a dark-adapted *S. mossambicus* injected with cytochalasin D (calculated final concentration, 4 μ g/ml) and transferred to light. Rods fail to elongate. Rod ellipsoid (R), outer limiting membrane (OLM). Bar, 10 μ m. \times 515.

that subunits add to the proximal free ends of the ellipsoid filaments. This scheme for assembly would be similar to the direction of assembly in regenerating microvilli (22). However, the possibility that monomer addition could take place at the calyx tip cannot be ruled out.

TABLE IV
Effect of Colchicine (A) and Cytochalasin B (B) on
Light-adapted Rods

	Rod inner segment length*
	μ m
A.	
Colchicine (calculated final concentration, 4 mM)	39.4 \pm 1.0
Control (L-15 buffer)	38.2 \pm 0.6
B.	
Injected eye + L-15 buffer	41.0 \pm 1.1
Injected eye + L-15 + DMSO	41.2 \pm 1.3
Cytochalasin B (calculated final concentration, 6 μ g/ml)	38.7 \pm 0.6

* Mean \pm SE; n = 30.

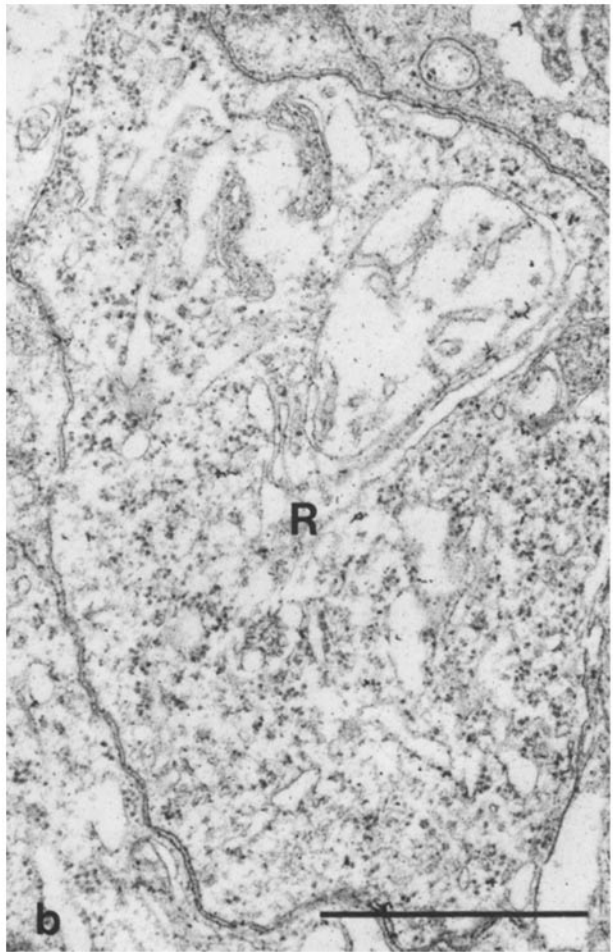
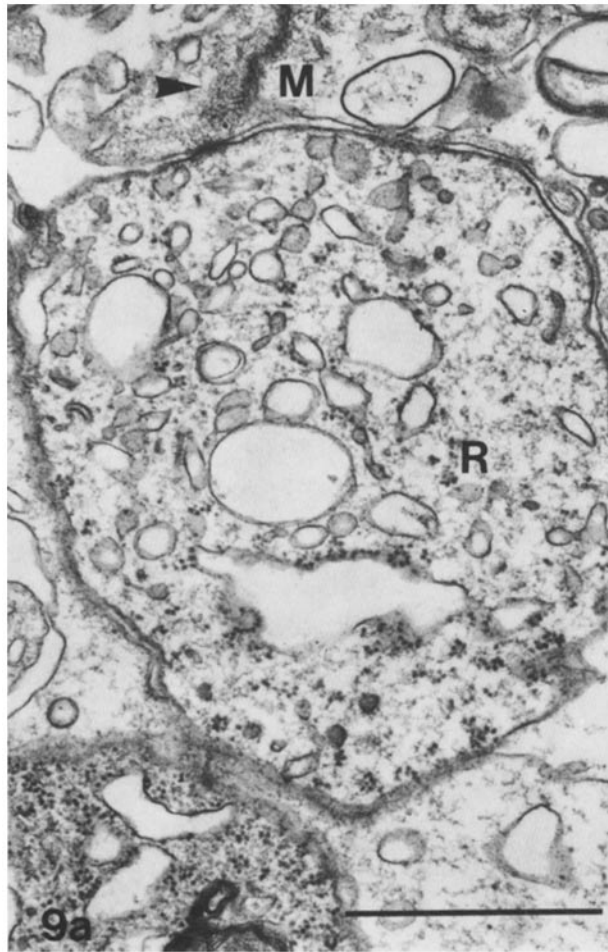


FIGURE 9 (a) Electron micrograph of a cross section of a dark-adapted rod myoid from *S. mossambicus*. Although the thin filaments (arrowhead) of the adherens junctions in adjacent Müller cells (M) are easily discernible, no obvious thin filaments are visible in the rod myoid (R). Bar, 1 μ m. \times 34,000. (b) Electron micrograph of a cross section of a rod myoid from *S. mossambicus* injected with Cytochalasin B. As in the case of the dark-adapted myoid, no obvious thin filaments are observed in the rod myoid (R). Bar, 1 μ m. \times 34,000.

We find that cytochalasins B and D reversibly inhibit light-induced elongation in *S. mossambicus* rods. Cytochalasin D is more potent than cytochalasin B in blocking elongation. This is consistent with the relative affinities of the two compounds for motility-related binding sites (16), their relative abilities to inhibit actin polymerization rates (7), and their relative effectiveness in disrupting events of motility (20).

Although the mechanism by which the cytochalasins act is not clearly understood, recent studies suggest that cytochalasins may inhibit actin assembly rates by interacting with filament ends (7, 18) or by binding to a complex that controls the formation and membrane attachment of microfilaments (16). It has also been suggested that the cytochalasins may directly inhibit actin filament-filament interactions, resulting in the weakening of an actin network (18). Because we do not see actin filaments in the myoids of cytochalasin-blocked rods, we think that cytochalasin is acting at the level of assembly. Our observations do not rule out the possibility that filament-filament interactions might also contribute to elongation by stabilizing or stiffening the actin filament array in the myoid.

Judging from the spatial and temporal occurrence of actin filaments within the rod myoid, our inhibitor studies, and the current information on the mode of action of the cytochalasins, we propose that rod elongation is produced by the assembly of actin filaments and possibly their cross-linking into rigid bundles. A similar mechanism has been proposed for the extension of the acrosomal filament in *Thyone* sperm (23, 24) and for the formation of microvilli after fertilization of sea urchin eggs (5).

We wish to thank Dr. Howard Bern and Richard Nishioka for generously supplying us with *S. mossambicus* during the course of this study, Dr. Greenfield Sluder for preparation of lumicolchicine, and Michael Nagata for excellent technical assistance.

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NOTE ADDED IN PROOF: In recent experiments using colchicine injections into dark-adapted eyes, we have found that in a few cases colchicine injections induced partial light-adaptive retinomotor movements in rods. Though we performed controls in the experiments reported in this paper, we cannot rule out the possibility that in some of our colchicine-injected fish, rods were in fact induced to elongate before microtubules were completely disrupted. Therefore, to ensure complete microtubule disruption before initiation of rod elongation, we have carried out an additional experimental series in which cold is used to disrupt rod microtubules before light-induced elongation. Isolated dark-adapted retinas were cultured in the presence or absence of 10^{-4} M Colcemid at 0°C for 30 min. During cold treatment all microtubules were completely abolished

($\bar{X} = 0.05$, $n = 22$) and no rod movement was observed. When cold-treated retinas were subsequently cultured at 22°C for 45 min in the light, rods elongated to similar lengths in Colcemid ($\bar{X} = 25.0 \pm 2.2$, $n = 40$) and Colcemid-free ($\bar{X} = 24.6 \pm 0.3$, $n = 40$) retinas. However, microtubules reappeared only in rod myoids of Colcemid-free cultures ($\bar{X} = 2.6$, $n = 27$ for Colcemid-free cultured retinas and $\bar{X} = 0.03$, $n = 33$ for Colcemid cultured retinas). Thus our results substantiate our hypothesis that rod elongation is microtubule independent.

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